

What is Claimed is:

1. A plasmid wherein two restriction enzyme recognition sites into which a T-vector can be cloned are introduced at the downstream of a promoter of a vector that is constantly expressed at high levels regardless of the kind of a host cell, whereby the plasmid functions as both the T-vector and an expression vector and has the property of allowing the expression of a target gene to be examined only by one-step T-vector cloning.
- 10 2. The plasmid according to claim 1, wherein the restriction enzyme recognition sites into which the T-vector can be cloned is selected from the group consisting of *HphI*, *MboII*, *AspEI* and *XcmI*, and a polynucleotide is inserted between the two restriction enzyme recognition sites.
- 15 3. The plasmid according to claim 2, wherein a nucleotide having thymine bases at both 3'-ends of the removal position of the inserted polynucleotide is exposed, when the plasmid is cut with the restriction enzymes.
- 20 4. The plasmid according to claim 1, wherein the constitutive high-level expression vector is pHCE.
- 25 5. A plasmid (pHCE-FOREX) functioning as both a T-vector and an expression vector, wherein two *AspEI* restriction enzyme recognition sites are introduced at the downstream of the HCE promoter of the pHCE vector, and a polynucleotide having *AspEI* restriction enzyme recognition sites at its both ends is inserted between the two *AspEI* restriction enzyme recognition sites.
- 30 6. A constitutive high-level expression T-vector (pHCE-FOREX-T), which is obtained by digesting the plasmid pHCE-FOREX of claim 5 with an *AspEI* restriction enzyme, to remove the polynucleotide having *AspEI* restriction enzyme

recognition sites at its both ends, and in which a nucleotide having thymine bases at both 3'-ends of the removal position of the polynucleotide is exposed.

7. A method for producing a plasmid (pHCE-FOREX) functioning as both a T-
5 vector and an expression vector, the method comprising the steps of:

- (a) constructing pHCE-M1 which the restriction enzyme recognition sites were removed by inducing point mutation in *Asp*EI restriction enzyme recognition sites in a pHCE vector;
- 10 (b) constructing pHCE-M2 by introducing two *Asp*EI restriction enzyme recognition sites into the downstream of the HCE promoter of the pHCE-M1 vector by PCR using primers containing the *Asp*EI restriction enzyme recognition sites; and
- 15 (c) inserting a polynucleotide having *Asp*EI restriction enzyme recognition sites at its both ends, between the two *Asp*EI restriction enzyme recognition sites of the pHCE-M2 vector.

8. An expression vector, which is obtained by digesting the plasmid of claim 2 with the restriction enzymes to remove the inserted polynucleotide, and then inserting a gene encoding a target protein, into a position from which the
20 polynucleotide was removed.

9. An expression vector, wherein a gene encoding a target protein is inserted into the constitutive high-level expression T-vector (pHCE-FOREX-T) of claim 6.

25 10. The expression vector according to claim 8 or 9, wherein the target protein-encoding gene is a gene amplified by PCR.

11. The expression vector according to claim 8 or 9, wherein the target protein-encoding gene is a PCR product amplified by using a primer having the amino
30 terminal end of ATG, and a primer specific to the base sequence of the gene.

12. The expression vector according to claim 8 or 9, wherein *NdeI* restriction enzyme recognition site is formed in the insertion position of the gene encoding the target protein.

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13. A microorganisms transformed with the expression vector of any one claim among claims 8 to 12.

10 14. A method for expressing a gene encoding target protein, which comprises culturing the transformed microorganisms of claim 13.

15. An expression vector library, which is prepared by the method comprising the steps of:

15 (a) removing the inserted polynucleotide by digesting the plasmid of claim 2 with the restriction enzyme selected from the group consisting of *HphI*, *MboII*, *AspEI* and *XcmI*; and

(b) inserting the library of various genes into a position from which the polynucleotide was removed.

20 16. An expression vector library wherein the library of various genes is inserted into the high-level expression T-vector (pHCE-FOREX-T) of claim 6.

17. A method for determining the cloning of a target gene, the method comprising the steps of:

25 (a) transforming microorganisms with the expression vector library of claim 15 or 16; and

(b) culturing the transformed microorganisms.

18. The method for determining the cloning of a target gene according to claim 17, wherein further comprising the steps of: separating a plasmid after the step (b); and digesting the plasmid with an *Nde*I restriction enzyme.